Current Topics in Biochemical Research
Vol. 14, *1*, 2012

Original Communication

p38 MAP kinase attenuates phorbol ester-induced ERK MAP kinase activation in adult cardiac ventricular myocytes

Benoit Boivin[1,2] and Bruce G. Allen[1,2,3,4,*]

[1]Montreal Heart Institute, 5000 Bélanger St., Montreal, Quebec, H1T 1C8, [2]Departments of Biochemistry and [3]Medicine, Université de Montréal, Montréal, Québec, H3C 3J7, [4]Department of Pharmacology and Therapeutics, McGill University, Montréal, Québec, H3G 1Y6, Canada

ABSTRACT

Cardiac hypertrophy may result from extracellular stimuli activating intracellular signalling pathways, including the mitogen-activated protein kinase (MAPK) pathways. The MAPK pathways include three cascades: ERK1/2, JNK, and p38. ERK1/2 is activated by mitogens whereas stress-related stimuli activate JNK and p38. In cardiac myocytes, activation of ERK1/2 by H_2O_2 is attenuated by p38. This study was to determine if p38 inhibits activation of ERK1/2 by PMA, a mitogen and potent activator of ERK1/2, in adult cardiac myocytes. Inhibition of p38α/β with SB203580 increased PMA-stimulated ERK1/2 and MEK1/2 phosphorylation. FPLC of myocyte lysates on MonoQ revealed two peaks of PMA-stimulated MBP kinase activity, ERK2 and ERK1. PD 98059, which blocks MEK1/2 activation, attenuated PMA-induced ERK1/2 activation. SB203580 did not increase ERK1/2 activity. Three peaks of PMA-stimulated MEK activity were detected following chromatography on Mono Q. Peaks 1, 2, and 3 eluted at 20-25 mM, 80-100 mM, and 210 mM NaCl, respectively. PD 98059 inhibited each of these activities. Peaks 2 and 3 were increased three-fold by SB203580. In addition, a new peak was detected eluting at 40-70 mM NaCl. Hence, in adult cardiac myocytes, p38α/β attenuates PMA-dependent MEK1/2 and ERK1/2 phosphorylation.

*Correspondence author
bruce.g.allen@umontreal.ca

KEYWORDS: cardiac myocyte, p38 MAP kinase, ERK1/2MAP kinase, cross talk

ABBREVIATIONS

ACVMs, adult cardiac ventricular myocytes; DMSO, dimethylsulfoxide; DTT, dithiothreitol; ERK, extracellular signal-regulated kinase; FPLC, fast protein liquid chromatography; GST, glutathione *S*-transferase; MAPK, mitogen activated protein kinase; MBP, myelin basic protein; PAGE, polyacrylamide gel electrophoresis; PKI, cyclic AMP-dependent protein kinase inhibitory peptide; PMA, phorbol 12-myristate 13-acetate; PMSF, phenylmethylsulfonyl fluoride; TX-100, Triton X-100

INTRODUCTION

Cardiac hypertrophy results from hemodynamic overload and/or increases in neurohormonal factors [1, 2]. Endothelin-1, angiotensin II, α_1-adrenergic agonists and phorbol esters all induce hypertrophy [3-8] and activate the Raf-MEK-ERK cascade. Hypertrophic growth of cardiac myocytes is associated with distinct changes in cell morphology and the pattern of gene expression. Constitutive activation of Ras [9, 10], Raf-1 [11], or MEK1 [12] is sufficient to induce a hypertrophic response. Interference with ERK activation using pharmacological inhibitors of MEK1/2, antisense oligonucleotides to ERK1/2, or expression of catalytically inactive Raf, MEK, ERK1, or the MAPK phosphatase CL100, can inhibit the hypertrophic response (reviewed in [13, 14]).

Hence, ERK activation appears to play an essential role in the hypertrophic response. However, whereas mice expressing constitutively activated Ras develop pathological hypertrophy [10], mice expressing activated MEK1 develop a compensated hypertrophy [12]. Interestingly, mice lacking ERK1 and having only one ERK2 allele showed a normal hypertrophic response [15]. Furthermore, transgenic mice in which ERK1/2 phosphorylation is prevented by overexpressing the dual specificity phosphatase 6 (DUSP6), an ERK1/2-specific phosphatase, showed a predisposition to fibrosis and apoptosis, in addition to a normal hypertrophic response to pressure overload or phenylephrine whereas ERK phosphorylation was prevented [15]. These results suggest that ERK1/2 activation may not be essential for cardiomyocyte hypertrophy and is actually cardioprotective. Hence, in addition to Raf-MEK-ERK, other pathways appear to be involved in hypertrophic signalling. p38 has been shown to regulate the phosphorylation of ERK1/2 in response to H_2O_2 [16], suggesting p38 pathway can crosstalk and modulate ERK1/2 signalling in response to redox signalling. The purpose of the present study was to determine if p38 influences the activation of ERK1/2 in adult cardiac ventricular myocytes in response to a mitogenic phorbol ester, phorbol 12-myristate 13-acetate (PMA).

MATERIALS AND METHODS

Materials

[γ-^{32}P]ATP was from GE Healthcare Canada. Antisera specific for pan-Ras (Ab-3) was from Calbiochem (San Diego, CA). Antibodies to MAPK-activated protein kinase 2 (MK-2, sc-6621) and Mek-1 (sc-219) was from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-Raf-1 antisera and GST-ERK1 (K71A) were from Dr. Steven Pelech (UBC, BC, Canada). Anti-ERK1-CT antiserum was raised against a synthetic peptide, corresponding to residues 333-367 of rat ERK1 MAP kinase (Global Peptide Services, Fort Collins, CO, USA) and recognized both ERK1 and ERK2. Recombinant canine hsp27, cloned into the pET24a expression vector [17], was a kind gift from Dr. William Gerthoffer, Reno, NV. Myelin basic protein (MBP) was purified from bovine brain as described previously [18]. Horseradish peroxidase-conjugated secondary antibodies were from Jackson ImmunoResearch Laboratories Inc. The enhanced chemiluminescence reagent Renaissance Plus was from PerkinElmer Life Sciences (Woodbridge, Ontario). cAMP-dependent protein kinase inhibitor peptide (PKI, amino acid sequence TTYADFIASGRTGRRNAIHD) was from the University of Calgary Peptide Synthesis Core Facility. Membrane-grade Triton X-100 (TX-100), leupeptin and PMSF were from Roche Applied Science (Laval, Québec). Microcystin LR, PD98059, and phorbol 12-myristate 13-acetate were from Calbiochem. SB203580 was a gift from Dr. John C. Lee (SmithKline Beecham, King of Prussia, PA). SDS-polyacrylamide gel electrophoresis reagents, nitrocellulose (0.22 µm), and Bradford protein assay reagents were from Bio-Rad Laboratories (Mississauga, Ontario). Fatty acid-free BSA and Tris base were from Sigma Chemical Co (Mississauga, Ontario). Unless otherwise stated, all other reagents were of analytical grade from VWR Canlab (Ville Mont-Royal, Québec). NanoPure-grade water was employed throughout these studies.

Isolation of rat cardiac ventricular myocytes

Adult rat cardiac ventricular myocytes (ACVM) were isolated as previously described [19]. This preparation provided 6 to 8 million cells/heart with 70 to 85% viability. Myocyte viability and purity were assessed by microscopy. No other cell types were detected.

Stimulation of myocytes and preparation of lysates

Adult ventricular myocytes were divided into 1 ml aliquots in 1.5 ml microcentrifuge tubes and used immediately after isolation. PMA, SB203580, and PD 98059 were added directly to the cell suspensions from 1000-fold stock solutions prepared in either 100% DMSO (PMA, PD 98059) or distilled water (SB203580). Cells were incubated with PD 98050 or SB203580 for 5 min at room temperature followed by stimulation with 10 nM PMA for 5 min. Cells were then chilled to 0°C for 1 min, centrifuged for 3 min at 2,000 rpm and 5°C, and resuspended in 0.5 ml of ice-cold lysis buffer (50 mM Tris-HCl, pH 7.5 at 5°C, 20 mM β-glycerophosphate, 20 mM NaF, 5 mM EDTA,

10 mM EGTA, 1 mM Na_3VO_4, 10 mM benzamidine, 0.5 mM PMSF, 10 µg/ml leupeptin, 5 mM DTT, 1 µM microcystin LR, and 1% v/v TX-100). Cells were extracted by mixing for 15 min at 5°C, centrifuged at 13,000 xg and 5°C for 15 min, and the soluble fraction retained. Lysates were aliquoted, frozen using liquid N_2, and stored at -80°C.

Electrophoresis and immunoblotting

SDS-PAGE and immunoblotting were performed as described previously [20].

FPLC of myocyte lysates

Lysates from ventricular myocytes were diluted with lysis buffer to a protein concentration of 10 mg/ml and recentrifuged (13,000 xg, 10 min, 5°C). Protein samples were applied, using a 0.5 ml sample loop, to a Mono Q HR 5/5 column equilibrated with 50 mM Tris-HCl (pH 7.4 at 5°C), 20 mM β-glycerophosphate, 2 mM EDTA, 2 mM EGTA, 5% (v/v) glycerol, 0.03% (v/v) Brij 35, 1 mM benzamidine, 1 µg/ml leupeptin, 1 mM Na_3VO_4, and 0.1% (v/v) β-mercaptoethanol. Following a 5 ml isocratic wash, proteins were eluted using a NaCl gradient (24 ml, 0-0.40 M NaCl; 0.1 ml, 0.40-1.0 M NaCl; 0.9 ml, 1.0 M NaCl) at a flow rate of 0.3 ml/min. Sixty fractions of 0.5 ml were collected. The fast protein liquid chromatography (FPLC) system was maintained in a chromatography cabinet at 5°C. Fractions 2-8 from the Mono Q column were pooled and applied to a Mono S HR 5/5 column previously equilibrated with 20 mM HEPES (pH 7.5 at 5°C), 20 mM β-glycerophosphate, 2 mM EDTA, 2 mM EGTA, 5% (v/v) glycerol, 0.03% (v/v) Brij 35, 1 mM benzamidine, 1 µg/ml leupeptin, 1 mM Na_3VO_4, and 0.1% (v/v) β-mercaptoethanol. Following a 5 ml wash, proteins were eluted using a NaCl gradient (24 ml, 0-0.40 M NaCl; 0.1 ml, 0.40-1.0 M NaCl; 0.9 ml, 1.0 M NaCl) at a flow rate of 0.4 ml/min. Seventy fractions of 0.5 ml were collected.

Assay of ERK activity

The activation of ERK was determined by its ability to phosphorylate myelin basic protein (MBP). Each FPLC fraction (20 µl) was assayed. Assays were for 45 min at 30 °C in a final volume of 30 µl in the presence of 50 mM Tris-HCl (pH 7.5 at 30°C), 13 mM β-glycerophosphate, 0.5 mg/ml MBP, 10 mM $MgCl_2$, 1.3 mM EDTA, 1.3 mM EGTA, 1.0 mM Na_3VO_4, 0.1 mM [γ-^{32}P]ATP (50 to 100 cpm/pmol), 1 µM PKI, 10 µg/ml leupeptin, and 10 mM DTT. Reactions were initiated by the addition of 10 µL of 3x assay media and terminated by spotting 20 µl onto 1.5 x 1.5 cm squares of P81 phosphocellulose paper that were immediately immersed in 0.5% H_3PO_4. Papers were washed three times for 5 min each in 500 ml of 0.5% H_3PO_4, dried, and ^{32}P incorporation was quantified by Cerenkov counting [21].

Assay of MEK and MAPKAP kinase 2 activities

Activation of the p38 MAPK cascade was assayed by measuring the activity of the downstream kinase, MAP kinase-activated protein kinase-2 (MK-2), using recombinant hsp27 as substrate. MEK activity was assayed by its ability to phosphorylate a specific substrate, GST-ERK1 (K71A). Indicated FPLC fractions (20 µl) were assayed as described previously [19, 22].

Miscellaneous methods

Protein concentration was measured by the Bradford [23] method using bovine γ-globulin as a standard.

RESULTS

There are numerous examples showing opposing effects of ERK1/2 and p38 MAPK. In addition, p38 MAPK activation decreases H_2O_2-induced ERK phosphorylation in adult cardiac ventricular myocytes (ACVMs), suggesting a crosstalk between the two cascades in which p38 exerts a negative regulation on the ERK pathway in redox signalling [16]. We sought to determine if p38 also modulated ERK signalling in response to a potent mitogen and hypertrophic agent, PMA. Increased phosphorylation of both ERK and MEK was observed in ACVMs pre-treated with SB203580 prior to application of PMA as compared to the levels produced by PMA alone (Fig. 1A).

To assess ERK activity, lysates were resolved on Mono Q and MBP phosphorylation determined. Treatment of myocytes with PMA activated myelin basic protein (MBP)-kinase activities that were resolved into 2 peaks (Fig. 1B). The MBP

phosphorylating activities in peaks 1 and 2 were inhibited by pre-treatment with PD 98059, an inhibitor of MEK activation. Antisera against ERK1 and ERK2 indicated that ERK1 was present in the fractions corresponding to both peaks 1 and 2 (Fig. 1C) whereas ERK2 immunoreactivity was only detected in peak 1. The lower band was confirmed as ERK2 using ERK2-specific antisera (not shown). Preincubation with a SB203580 failed to increase ERK activity towards MBP.

To determine the efficacy of the treatment with SB203580, activation of the p38 MAPK cascade was assayed by measuring the activity of the downstream kinases, MAP kinase-activated protein kinases -2, -3 and -5 (MK-2, MK-3, MK-5), using recombinant hsp27 as substrate. Hsp27-phosphorylating activity was observed in unstimulated cardiac ventricular myocytes (Fig. 2). Tandem ion exchange chromatography resolved

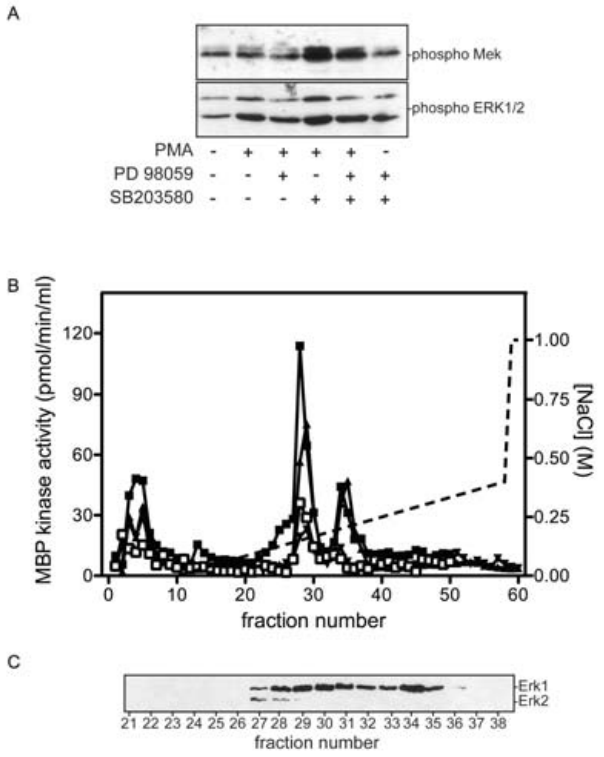

Fig. 1. Effect of SB203580 on PMA-induced ERK phosphorylation and kinase activity. (A) Phospho ERK1/2 and phospho MEK1/2 immunoblot. ACVMs were treated as indicated, lysates prepared and ERK and MEK phosphorylation assessed by immunoblotting. **(B)** Mono Q profile of MBP phosphorylation. Freshly isolated ACVMs were treated with vehicle (□), PMA (5 min, 10 nM; ■), PD98059 (10 µM, 5 min) followed by PMA (5 min, 10 nM; ▼), or SB203580 (10 µM, 5 min) followed by PMA (5 min, 10 nM; ▲). Lysates were prepared, resolved on a Mono Q HR 5/5 column, and MBP phosphorylation was determined. **(C)** ERK1/2 immunoblot. Aliquots (40 µl) of fractions 21-38 from figure 1B were resolved on SDS-PAGE, transferred to nitrocellulose membranes and probed with an anti-ERK1/2 antibody. No bands other than those shown were detected.

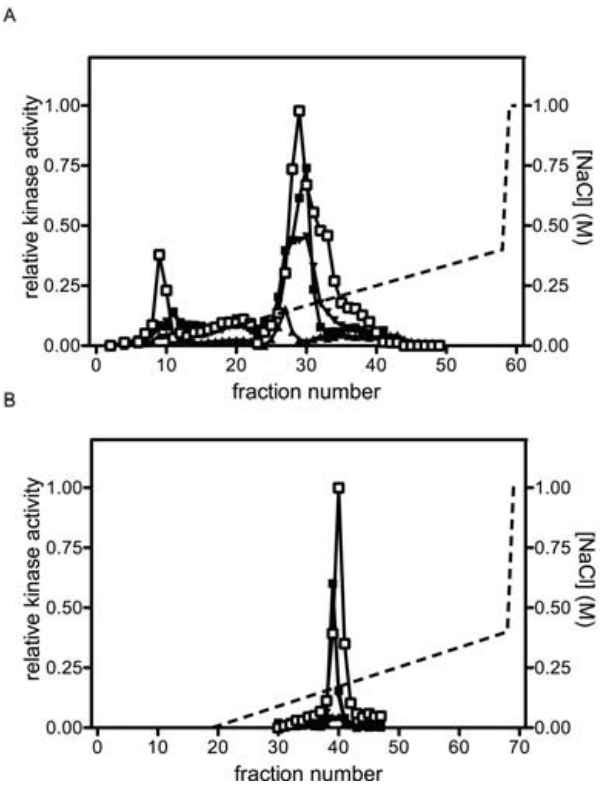

Fig. 2. The p38 MAPK cascade is activated in freshly isolated cardiac myocytes. Activation of the p38 MAPK cascade was assayed by measuring the activity of the downstream kinases, MAP kinase-activated protein kinase-2 (MK2), MK3 and MK5, using recombinant hsp27 as substrate. Freshly isolated ACVMs were treated with vehicle (□), PMA (5 min, 10 nM; ■), PD98059 (10 µM, 5 min) followed by PMA (5 min, 10 nM; ▼), or SB203580 (10 µM, 5 min) followed by PMA (5 min, 10 nM; ▲) as described in methods. Fraction 2-8 from the Mono Q column were pooled and rechromatographed on Mono S. Hsp27-phosphorylating activity was observed in the elution profiles following both Mono Q (**A**) and Mono S (**B**) chromatography. Values in both A and B have been normalized to the highest level of activity measured within the two data sets.

several peaks of hsp27-phosphorylating activity: all of which were inhibited by SB203580, confirming all hsp27-kinase activities are downstream of p38α/β. No corresponding peaks of activity were observed following chromatography of lysates from whole heart (not shown), suggesting this activation likely resulted from the stresses of the cell isolation procedure.

ERK is activated by direct phosphorylation by upstream MAPK kinases: MEK1 and MEK2. Hence, to determine the point in the ERK1/2 cascade at which p38 MAPK was acting to regulate ERK1/2 phosphorylation, MEK activity was determined by screening fractions from the Mono Q column for ERK-phosphorylating activity. In total, 5 peaks of ERK1-phosphorylating activity were observed (Fig. 3). Three peaks of PMA-stimulated MEK activity were detected; peaks 2, 4, and 5 eluted at 20-25 mM, 80-100 mM, and 210 mM NaCl, respectively. Pre-treatment of intact myocytes with PD 98059 inhibited each of these activities. Unexpectedly, pre-treatment with SB203580 increased ERK1-phosphorylating activities eluting in peaks 1, 4, and 5. Peak 2 remained unaffected. In addition, a new peak of activity, peak 3, was detected eluting at 40-70 mM NaCl. To determine if the enhanced ERK-phosphorylating activity evoked by pre-treatment with SB203580 represented activation of MEK, immune complex assays were performed. Antisera for MEK1 specifically immunoprecipitated the ERK1-phosphorylating activity in fractions corresponding to both peak 2 and peak 4 (data not shown). These results confirm that inhibition of p38 MAPK increased MEK1 activation and suggest that p38 MAPK activity suppresses MEK1/2 activity.

Finally, we examined if a MAPKKK activity was altered upon inhibition of p38 MAPK. A GST-MEK1 pull-down assay followed by MEK1 phosphorylation revealed that PMA activated MEKK activity (Fig. 4). This kinase activity was inhibited by the MEK inhibitor PD 98059 whereas inhibiting p38 using SB203580 produced an increase in MAPKKK activity. In contrast, although no PMA-activated MEKK activity was detected in Ras immune complexes, pre-treatment with SB203580 resulted in the appearance of a MEK1 kinase activity in the Ras immune-complexes.

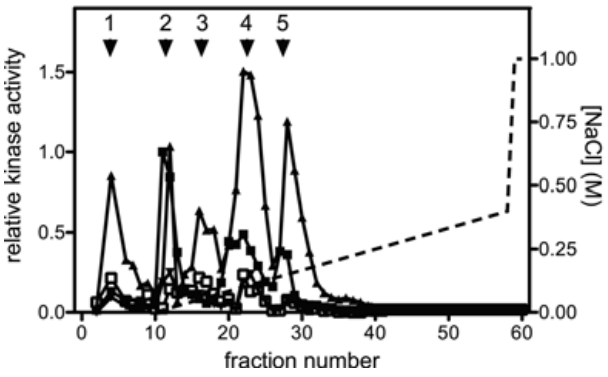

Fig. 3. SB203580 increases PMA-stimulated MEK activity. Freshly isolated ACVMs were treated with vehicle (□), PMA (5 min, 10 nM; ■), PD98059 (10 µM, 5 min) followed by PMA (5 min, 10 nM; ▼), or SB203580 (10 µM, 5 min) followed by PMA (5 min, 10 nM; ▲). Lysates were prepared, resolved on a Mono Q HR 5/5 column, and MEK activity was determined by measuring the phosphorylation of a kinase-inactive mutant of ERK1, GST-ERK1(K71A). Values have been normalized to the highest level of activity measured within the data set.

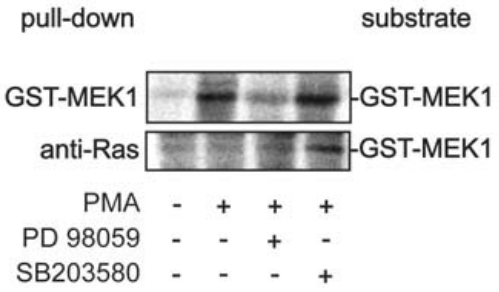

Fig. 4. SB203580 stimulates MEK kinase activity. Lysates were prepared from stimulated myocytes as described under Materials and Methods. Ras or MEK kinase were 'pulled down' by incubating 100 µg of each sample with either 10 µl protein A agarose pre-coupled with 2 µg of an anti-Ras antibody or 1 µg of GST-MEK1 (K97A) conjugated to glutathione Sepharose 4B. Complexes were washed 4 times with 500 µl of ice-cold buffer (50 mM Tris-HCl, pH 7.4 at 5°C, 150 mM NaCl, 20 mM β-glycerophosphate, 2 mM EDTA, 2 mM EGTA, 5% (v/v) glycerol, 0.03% (v/v) Brij 35, 1 mM benzamidine, 1 µg/ml leupeptin, 1 mM Na_3VO_4, 0.1% (v/v) β-mercaptoethanol and 1% TX-100). Complexes were then suspended in 20 µl of buffer and MEK kinase activity assayed, using recombinant kinase-inactive GST-MEK1 (K97A).

DISCUSSION

In the present paper we show that inhibition of p38α/β activity with SB203580 increased the phosphorylation of ERK1/2 and MEK1/2 in response to phorbol ester. This was accompanied by increased MEK and MEKK activity; however, the increased ERK phosphorylation appeared partially uncoupled from its catalytic activity as, although ERK phosphorylation increased, its catalytic activity did not.

Cross talk between the ERK1/2 and p38 pathways has been shown in numerous cell systems. In ACVMs, inhibition of p38α/β resulted in increased H_2O_2-stimulated ERK phosphorylation, and this was at least in part a result of an increased association of PP2A with ERK and MEK [16]. In RAW 264.7 cells stimulated with lipopolysaccharide (LPS) [24], and in osteosarcoma cells cultured in the presence of FBS [25], inhibition of p38 activity increased ERK phosphorylation. Similarly, in PC12 cells treated with epidermal growth factor, SB203580 increased ERK and MEK phosphorylation [26]. However, this cross talk between the two cascades appears to be stimulus specific. For example, it has also been reported in embryonic chick joint articular surface cells that inhibition of p38 increased early strain-induced ERK and MEK phosphorylation [27]. On the other hand, inhibition of p38 has no effect on ERK phosphorylation in response to fibroblast growth factor 2 or the PTP inhibitor pervanadate in the same cellular model. Similarly, in HUVECs, inhibiting p38α/β-enhanced IL-1α-induced ERK and MEK phosphorylation but reduced thrombin-stimulated MEK and ERK phosphorylation [28]. IL-1α or SB203580 activated Raf-1 whereas thrombin did not. Furthermore, SB203580 increased the activation of Raf induced by IL-1α. In contrast, whereas thrombin alone did not alter RAF activation, the presence of thrombin potentiated the effects of SB203580 upon RAF activity. Hence the ability of p38 to inhibit ERK1/2 phosphorylation depends upon the nature of the stimulus activating the ERK cascade. Furthermore, as the nature of the stimulus activating p38 alters which p38 isoforms become activated and the extent of p38 activation [29], the ability of the p38 pathway to regulate ERK phosphorylation may also depend upon the activation status of the different p38 isoforms. One possible explanation for this inhibitory effect is non-selectivity of the pharmacological inhibitors of p38 activity. However, expression of a dominant negative mutant of p38 also increased ERK1/2 phosphorylation in response to UVA [30]. Hence, cross talk between ERK and p38 pathways is ubiquitous but appears to differ depending upon the nature of the cellular stimulus.

Previous studies of p38-ERK cross talk examined ERK phosphorylation and, in some cases, downstream events that may be attributed to more than just changes in ERK activity. In the present study we assessed both ERK phosphorylation and ERK activity and found that although ERK phosphorylation was increased following pre incubation with SB203580, its activity did not increase. This raises the possibility of inhibitory phosphorylation sites within ERK itself that regulate the coupling between ERK phosphorylation and ERK activity.

CONCLUSIONS

The p38 MAPK pathway attenuates the activation of the ERK pathway in response to mitogenic stimuli in adult ventricular myocytes. This effect may be exerted at one or more steps in the regulation of Ras/Raf/MEK activation.

ACKNOWLEDGMENTS

We thank Dominique Chevalier for technical assistance. This work was supported by a grant from the Canadian Institutes of Health Research (FRN 77791). BGA was a New Investigator of the Heart and Stroke Foundation of Canada and Senior Scholar of the Fondation de la Recherche en Santé du Québec (FRSQ). BB was the recipient of a bursary from the Fondation de la Recherche en Santé du Québec (FRSQ).

CONFLICT OF INTEREST

There exists no potential conflict of interest.

REFERENCES

1. Cooper, G., IV. 1997, Ann. Rev. Med., 48, 13.
2. Swynghedauw, B. 1999, Physiol. Rev., 79, 215.
3. Shubeita, H. E., McDonough, P. M., Harris, A. N., Knowlton, K. U., Glembotski, C. C., Brown, J. H., and Chien, K. R. 1990, J. Biol. Chem., 265, 20555.

4. Ito, H., Hirata, Y., Hiroe, M., Tsujino, M., Adachi, S., Takamoto, T., Nitta, M., Taniguchi, K., and Marumo, F. 1991, Circ. Res., 69, 209.
5. Sadoshima, J. and Izumo, S. 1993, Circ. Res., 73, 413.
6. Sadoshima, J. and Izumo, S. 1993, Circ. Res., 73, 424.
7. Dunnmon, P. M., Iwaki, K., Henderson, S. A., Sen, A., and Chien, K. R. 1990, J. Mol. Cell. Cardiol., 22, 901.
8. Allo, S. N., McDermott, P. J., Carl, L. L., and Morgan, H. E. 1991, J. Biol. Chem., 266, 22003.
9. Thorburn, A., Thorburn, J., Chen, S. Y., Powers, S., Shubeita, H. E., Feramisco, J. R., and Chien, K. R. 1993, J. Biol. Chem., 268, 2244.
10. Zheng, M., Dilly, K., Dos Santos Cruz, J., Li, M., Gu, Y., Ursitti, J. A., Chen, J., Ross, J., Jr., Chien, K. R., Lederer, J. W., and Wang, Y. 2004, Am. J. Physiol., 286, H424.
11. Thorburn, J., McMahon, M., and Thorburn, A. 1994, J. Biol. Chem., 269, 30580.
12. Bueno, O. F., De Windt, L. J., Tymitz, K. M., Witt, S. A., Kimball, T. R., Klevitsky, R., Hewett, T. E., Jones, S. P., Lefer, D. J., Peng, C. F., Kitsis, R. N., and Molkentin, J. D. 2000, EMBO J., 19, 6341.
13. Wang, Y. 2007, Circulation, 116, 1413.
14. Lorenz, K., Schmitt, J. P., Vidal, M., and Lohse, M. J. 2009, Int. J. Biochem. Cell Biol., 41, 2351.
15. Purcell, N. H., Wilkins, B. J., York, A., Saba-El-Leil, M. K., Meloche, S., Robbins, J., and Molkentin, J. D. 2007, Proc. Natl. Acad. Sci. USA, 104, 14074.
16. Liu, Q. and Hofmann, P. A. 2004, Am. J. Physiol., 286, H2205.
17. Larsen, J. K., Gerthoffer, W. T., Hickey, E., and Weber, L. A. 1995, Gene, 161, 305.
18. Chevalier, D. and Allen, B. G. 2000, Prot. Exp. Purific., 18, 229.
19. Chevalier, D. and Allen, B. G. 2000, Biochemistry, 39, 6145.
20. Boivin, B., Chevalier, D., Villeneuve, L. R., Rousseau, E., and Allen, B. G. 2003, J. Biol. Chem., 278, 29153.
21. Allen, B. G., Andrea, J. E., and Walsh, M. P. 1994, J. Biol. Chem., 269, 29288.
22. Chevalier, D., Thorin, E., and Allen, B. G. 2000, J. Pharmacol. Toxicol. Meth., 44, 429.
23. Bradford, M. M. 1976, Anal. Biochem., 72, 248.
24. Xiao, Y. Q., Malcolm, K., Worthen, G. S., Gardai, S., Schiemann, W. P., Fadok, V. A., Bratton, D. L., and Henson, P. M. 2002, J. Biol. Chem., 277, 14884.
25. Shimo, T., Matsumura, S., Ibaragi, S., Isowa, S., Kishimoto, K., Mese, H., Nishiyama, A., and Sasaki, A. 2007, J. Cell Commun. Signal., 1, 103.
26. New, L., Li, Y., Ge, B., Zhong, H., Mansbridge, J., Liu, K., and Han, J. 2001, J. Cell Biochem., 83, 585.
27. Lewthwaite, J. C., Bastow, E. R., Lamb, K. J., Blenis, J., Wheeler-Jones, C. P., and Pitsillides, A. A. 2006, J. Biol. Chem., 281, 11011.
28. Houliston, R. A., Pearson, J. D., and Wheeler-Jones, C. P. D. 2001, Am. J. Physiol., 281, C1266.
29. Remy, G., Risco, A. M., Inesta-Vaquera, F. A., Gonzalez-Teran, B., Sabio, G., Davis, R. J., and Cuenda, A. 2010, Cell. Signal., 22, 660.
30. Zhang, Y., Zhong, S., Dong, Z., Chen, N., Bode, A. M., and Ma, W. 2001, J. Biol. Chem., 276, 14572.